

# Assessment of PAHs accumulation in *Donax trunculus* (Linnaeus, 1758) (Bivalvia, Donacidae) from the Bulgarian Black Sea Coast

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## Abstract

Anthropogenic pollution of marine ecosystems is one of the main sources of polycyclic aromatic hydrocarbons (PAHs). Marine bivalves are often used as bioindicators of environmental pollution due to their wide distribution and capability of xenobiotic bioaccumulation. The aim of the present study was to assess the presence of PAHs in soft tissues of wedge clams *Donax trunculus* (Linnaeus, 1758), collected from sublittoral sandy habitats at different locations off the Bulgarian Black Sea coast. Wedge clams from the different locations showed variations in the content of accumulated PAHs' compounds. The concentrations of PAHs were measured by gas chromatography system with mass spectrometry detection. The total PAHs content (sum of 13 PAHs' compounds) measured was in the range from 5.59 to 50.50 ng/g wet weight and was comparable with other European studies. The compounds phenanthrene and fluorene were most abundant in all analyzed samples. The results showed that low molecular weight (LMW) PAHs (2 and 3 aromatic rings) were predominant, accounting for 91% of the total PAHs levels, while high molecular weight (HMW) PAHs (4–5- and 6- rings) presence was 8.9% on average. The ratio LMW/HMW PAHs was higher than one, suggesting predominant pollution of petrogenic origin. The concentrations of benzo (a)pyrene did not exceed the limit set in EC Regulation although it was detected in 20% of the analyzed samples. In conclusion, maximum overall PAHs content was found in clams from Arkutino, while minimum PAHs content was present in samples from Elenite. The Sum PAH4 (sum of four polycyclic aromatic hydrocarbons: benzo[a]pyrene, chrysene, benzo[a]anthracene, and benzo[b]fluoranthene) in the

wedge clams for all localities studied was below legislation limits. Data from the present research can be used for assessing pollution levels in the marine environment and also risk of human exposure to PAHs using *D. trunculus* as bioindicator species.

### Keywords

bioaccumulation, Bulgaria, coastal marine ecosystems, polycyclic aromatic hydrocarbons, wedge clam

## Introduction

Pollution with polycyclic aromatic hydrocarbons (PAHs) is becoming a serious problem in marine ecosystems. PAHs are a group of organic compounds that consist of two, three or more condensed aromatic rings. They are highly persistent and widespread in the marine environment. PAHs are produced primarily from organic combustion and also from other anthropogenic activities and can enter into the marine environment in two main ways - by chronic pollution (associated with boat traffic) or by acute pollution due to oil spills (Kucuksezgin et al. 2020). Different marine bivalves (clams, oysters, mussels, scallops) have been recommended as suitable bioindicators of contamination in the sea due to their wide geographical distribution, filter feeding and rapid accumulation of toxic substances in their tissues (Suárez et al. 2013; Tlili and Mouneyrac 2019). Reliable data exist on the bioavailability and accumulation of marine environmental pollutants in bivalve tissues (such as PAHs, polychlorinated biphenyls, pesticides and heavy metals) and their toxic effects (Tanabe and Subramanian 2006; Georgieva et al. 2016; Lehtonen et al. 2019). However, the accumulation of organic pollutants depends not only on the physico-chemical characteristics of contaminants, but also on bivalve physiology and their lipid content (Barhoumi et al. 2016; Lehtonen et al. 2019). The potential of PAHs to cause adverse effects as endocrine- and reproductive disruption, genotoxicity and oxidative damage in marine organisms has been previously reported (Banni et al. 2010; Machado et al. 2014) and it was also demonstrated that acute benzo[a]pyrene exposure significantly depressed acetylcholinesterase (AChE) activity in gills and digestive gland of mussels (*Mytilus galloprovincialis*) (Banni et al. 2010).

The wedge clam (*D. trunculus*) is a species inhabiting fine sandy habitats of the upper infralittoral subzone and feeds by filtration on phytoplankton and suspended particulate matter. In the Bulgarian Black Sea coastal zone *D. trunculus* dominates usually between 1.0 and 6.5 m depth and is exposed to intense wave action and fluctuations of abiotic environmental factors (Gumus et al. 2020). Although local people do not traditionally consume wedge clams, *D. trunculus* are increasingly being collected with dredges for export due to the high prices on the foreign markets (Gumus et al. 2020). Data on the accumulation of PAHs in tissues of marine bivalve species is scarce, which determines the importance of research of PAHs' content accumulated in wedge clams inhabiting the Bulgarian Black Sea area.

The aim of the present study was to carry out the first comprehensive assessment of the levels of bioaccumulated PAHs in tissues of *D. trunculus* as an indicator of the pollution affecting the Bulgarian Black Sea coastal area and the possible risks for humans.

## Materials and methods

### Sampling and sample preparation

Wedge clams *D. trunculus* were collected manually or were obtained from commercial providers from their sublittoral sandy habitats along the Bulgarian Black Sea coast from May 2019 – September 2020 (Table 1). From each sampling location 2–3 kg of adult wedge clams of similar shell length (mean  $2.1 \pm 0.34$  cm) were gathered, placed in plastic bags, kept in ice and transported to the laboratory. Soft tissues of the individual wedge clams were removed and 250 g samples were formed. The tissues were homogenized and stored at  $-20$  °C until analysis.

### Chemical analysis

Analytical procedures used for preparing soft tissue of mussels were described previously (Georgieva et al. 2016) with some modification. Ten grams from the homogenized soft tissue of clams were taken for extraction. Each sample was mixed with anhydrous sodium sulfate in a mortar and spiked with internal standards PCB 30 and PCB 204. The compounds were extracted with hexane/dichloromethane (2:1; v/v) in Soxhlet Extractor for 16 h at a rate of five cycles per hour. The extract was cleaned-up on a multilayer glass column filled with neutral and acid silica. PAH compounds were eluted with n-hexane followed by n-hexane/dichloromethane (4:1 v/v). The eluates were concentrated

**Table 1.** Locations and period of *D. trunculus* collection along the Bulgarian Black Sea coast with GPS coordinates, depth and distance from the shore.

Code	Location	Period	GPS coordinates	Depth [m]	Distance from the shore [m]
S1	Varna Bay	May 2019	43.2667°N, 28.0266°E	1.50–2.00	50–100
S2	Kranevo	July 2019	43.2667°N, 28.0266°E	3.00–3.50	50–100
S3	Shkorpilovtsi	July 2019	42.9603°N, 27.8970°E	3.00–3.50	20–50
S4	Ahtopol	July 2019	42.1022°N, 27.9328°E	1.50–2.00	20–50
S5	Varna Bay	October 2020	43.2365°N, 28.0160°E	1.50–2.00	50–100
S6	Shkorpilovtsi	March 2020	42.9603°N, 27.8970°E	1.50–2.00	20–50
S7	Ahtopol	March 2020	42.1140°N, 27.9243°E	1.50–2.00	20–50
S8	Sveti Vlas	March 2020	42.7090°N, 27.7595°E	3.00–3.50	10–20
S9	Slanchev Bryag	March 2020	42.6906°N, 27.7137°E	1.50–2.00	5–10
S10	Irakli	May 2020	42.7498°N, 27.8901°E	3.00–3.50	100–150
S11	Nessebar	June 2020	42.6559°N, 27.7171°E	1.50–2.00	50–100
S12	Arkutino	June 2020	42.3311°N, 27.7368°E	2.50–3.00	50–100
S13	Duni	June 2020	42.3684°N, 27.7094°E	1.50–2.00	5–10
S14	Sozopol	June 2020	42.4148°N, 27.7004°E	1.50–2.00	5–10
S15	Primorsko	June 2020	42.2530°N, 27.7533°E	1.50–2.00	50–100
S16	Cape Emine	August 2020	42.7018°N, 27.8343°E	2.50–3.50	200–250
S17	Tsarevo	September 2020	42.1666°N, 27.8533°E	2.50–3.00	50–100
S18	Elenite	September 2020	42.7025°N, 27.8130°E	3.50–4.00	200–250
S19	Primorsko	September 2020	42.2577°N, 27.7520°E	1.50–2.00	50–100
S20	Arkutino	September 2020	42.3282°N, 27.7496°E	3.00–3.50	100–150

to near dryness with a gentle stream of nitrogen and reconstituted in 0.5 cm<sup>3</sup> in hexane. One microliter of extract was injected into the gas chromatography system in triplicate.

The quantitative analysis of PAHs was performed on gas chromatograph (GC/MS) GC FOCUS (Thermo Electron Corporation, USA) using POLARIS Q Ion Trap mass spectrometer (MS), equipped with an AI 3000 autosampler and splitless Injector. The experimental MS parameters, temperature of ion source and temperature of transfer line, were 220 °C and 250 °C, respectively. The PAHs experimental oven temperature was programmed as follows: 40 °C (1 min), 40 °C/min to 130 °C (3 min), 12 °C/min to 180 °C, 7 °C/min to 280 °C, 10 °C/min to 310 °C with a final hold for 5.0 min. The separation of compounds was achieved with a TG-5 ms capillary column with a length of 30 m, 0.25 mm ID and a film thickness of 0.25 µm (Thermo Fisher Scientific). The carrier gas helium was used at a flow rate of 1 ml/min.

## Quality control

Pure reference standard solution (EPA 525 PAH Mix B, 500 µg/mL) of each component in acetone (Supelco) was used for instrument calibration, quantification of compounds and recovery determination. Procedural blanks were analyzed between each 5 samples to monitor possible laboratory contamination.

In the prepared extracts thirteen PAHs compound were measured: acenaphthylene (ACL), anthracene (AN), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbFA), benzo[k]fluoranthene (BkFA), benzo[ghi]perylene (BghiP), benzo[a]pyrene (BaP), chrysene (CHR), dibenzo[a,h]anthracene (DbahA), fluorene (FL), indeno[1,2,3-cd]pyrene (IP), phenanthrene (PHE), pyrene (PY). Each sample was analyzed three times and an average was taken.

Limits of detection (LOD) were estimated as 3 times the standard deviation, based on the low concentrations of the analytes in the sample. LOD varied for individual compounds from 0.075 to 0.294 ng/g wet weight (ww). For each PAHs were (ng/g ww): ACL 0.185, FL 0.128, PHE 0.092, AN 0.294, PY 0.173, BaA 0.219, CHR 0.185, BbFA 0.155, BkFA 0.178, BaP 0.295, IP 0.175, DBahA 0.199, and BghiP 0.075. The concentrations were reported as MEAN±SD of three measurements. Differences in means were determined by t-test ( $p < 0.05$  considered significant).

## Results

The mean concentrations of individual PAHs measured ranged from 0.05 (benzo[g,h,i]perylene) to 19.03 ng/g ww (phenanthrene) (Table 2). Acenaphthylene, anthracene, benzo[a]anthracene, indeno[1,2,3-cd]pyrene and dibenzo[a,h]anthracene showed levels below the instrumental detection limit in all samples (not present in Table 2). Four of the 13 target PAHs were found in almost all samples. BkFA and BghiP were detected in clams with very low values, close to the limit of quantitation (LOQ). Phenanthrene (PHE) predominated (from 3.24 to 48.22 ng/g) in all samples.

**Table 2.** Individual PAHs concentrations (ME  $\pm$ SD ng/g ww) in *D. trunculus* from the representative localities off the Bulgarian Black Sea coast in different sampling periods (nd – not detected, PAHs: polycyclic aromatic carbons; BbFA: benzo[b]fluoranthene, BkFA: benzo[k]fluoranthene, BghiP: benzo[ghi]perylene, BaP: benzo[a]pyrene, CHR: chrysene, FL: fluorene, PHE: phenanthrene and PY: pyrene)\*.

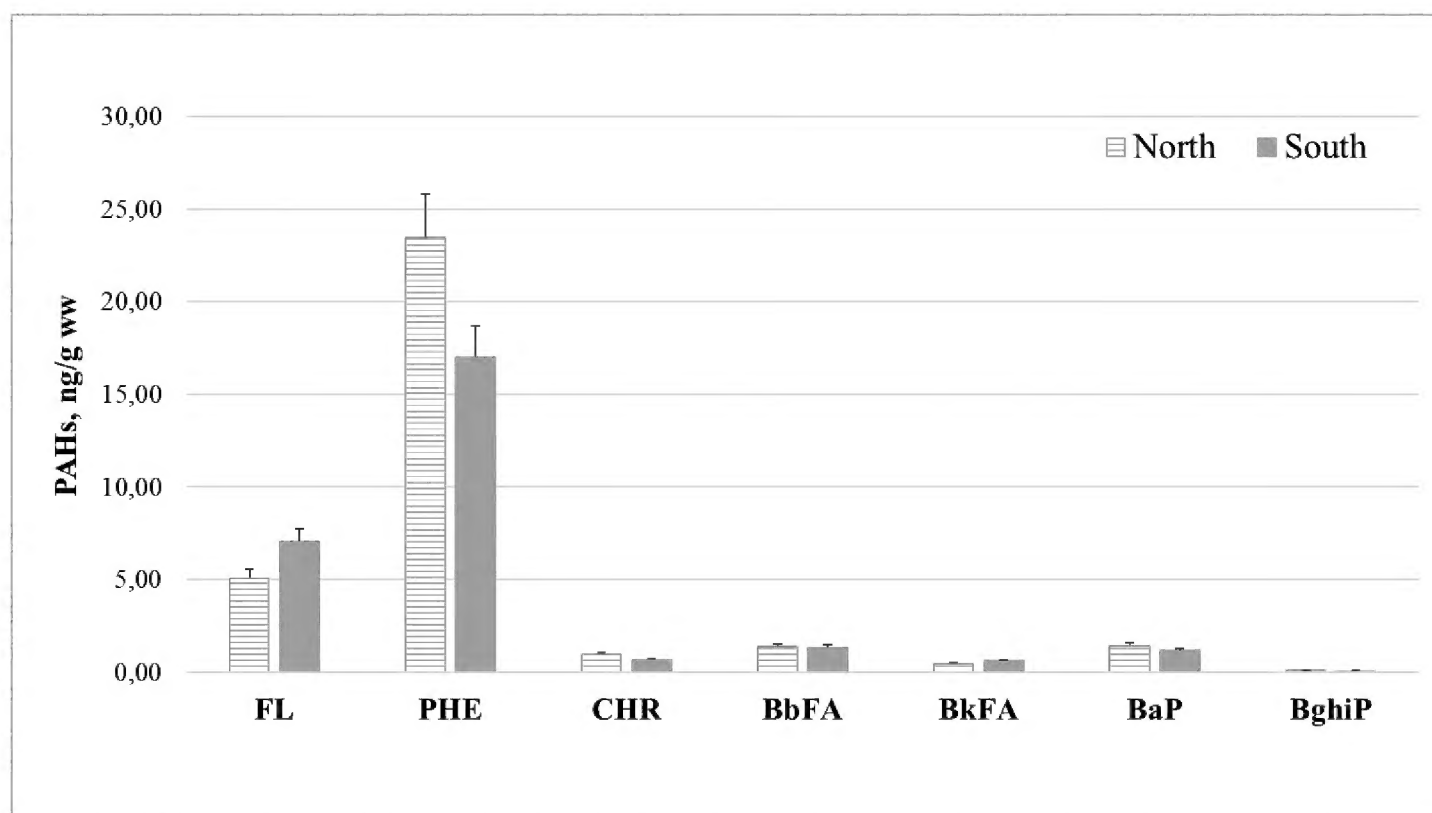
Site	Location	Period	FL	PHE	PY	CHR	BbFA	BkFA	BaP	BghiP	Sum PAH4	Sum PAH13
S1	Varna Bay	May-19	4.11	21.81	nd	0.92	nd	nd	nd	nd	0.92	26.84
S2	Kranevo	Jul-19	6.48	29.05	nd	nd	0.50	nd	0.54	nd	1.04	36.56
S3	Shkorpilovtsi	Jul-19	9.55	27.44	nd	nd	nd	0.46	nd	nd	0.00	37.44
S4	Ahtopol	Jul-19	0.43	3.24	nd	0.98	3.41	nd	nd	0.07	4.39	8.13
S5	Varna Bay	Oct-20	1.49	Nd	nd	nd	3.59	nd	2.34	0.07	5.93	7.49
S6	Shkorpilovtsi	Mar-20	4.57	18.10	nd	0.92	0.16	nd	nd	0.07	1.08	23.82
S7	Ahtopol	Mar-20	1.56	8.92	nd	0.90	1.36	nd	nd	nd	2.26	12.74
S8	Sveti Vlas	Mar-20	10.02	15.25	nd	0.53	0.30	0.54	nd	0.00	0.83	26.64
S9	Slanchev Bryag	Mar-20	1.97	8.17	nd	0.91	1.46	nd	1.08	nd	3.45	13.58
S10	Irakli	May-20	7.71	35.31	0.30	1.10	1.33	nd	nd	0.08	2.43	45.83
S11	Nessebar	Jun-20	14.21	31.19	nd	0.28	3.38	nd	nd	nd	3.66	49.07
S12	Arkutino	Jun-20	0.21	48.22	nd	0.56	0.21	nd	1.22	0.07	2.00	50.50
S13	Duni	Jun-20	21.00	22.68	nd	0.64	0.24	nd	nd	nd	0.89	44.57
S14	Sozopol	Jun-20	4.51	nd	nd	0.41	2.13	0.81	nd	nd	2.54	7.86
S15	Primorsko	Jun-20	6.12	7.17	nd	0.46	0.26	nd	nd	0.10	0.72	14.11
S16	Cape Emine	Aug-20	2.99	19.72	nd	0.47	1.18	0.42	nd	0.08	1.65	24.84
S17	Tsarevo	Sep-20	9.38	73.81	nd	nd	4.10	nd	nd	0.00	4.10	87.29
S18	Elenite	Sep-20	0.60	4.16	nd	nd	0.78	nd	nd	0.06	0.78	5.59
S19	Primorsko	Sep-20	nd	5.62	nd	nd	1.76	nd	nd	0.00	1.76	7.38
S20	Arkutino	Sep-20	0.63	20.38	nd	0.64	0.56	nd	nd	nd	1.21	22.21

\*Acenaphthylene, anthracene, benzo[a]anthracene, indeno[1,2,3-cd]pyrene and dibenzo[a,h]anthracene showed levels below the instrumental detection limit in all samples and are not present in the Table.

The sum of 4 PAHs: benzo[a]pyrene, chrysene, benzo[a]anthracene, and benzo[b]fluoranthene (Sum PAH4) in the studied wedge clams from the Bulgarian Black Sea coastal zone varied in the range from 0.72 to 5.93 ng/g ww (Table 2). Relatively low Sum PAH4 levels were observed in wedge clams from 3 sampling locations (Shkorpilovtsi (S3), Primorsko (S15) and Elenite (S18)) which are situated far from highly urbanized and industrial areas off the Black Sea coast (Table 2). In contrast, the Sum PAH4 in *D. trunculus* from Varna Bay (S5) (industrial city and harbor area) was found to be higher (5.93 ng/g ww) (Table 2). The total PAHs levels (sum of 13 individual PAHs, Sum PAH13) in samples from Elenite (S18) (5.59 ng/g ww) and Tsarevo (S17) (87.29 ng/g ww) differed significantly ( $p < 0.05$ ). Relatively high total PAH levels were observed also in wedge clam samples from Arkutino (S12), Nessebar (S11), Irakli (S10), and Duni (S13).

Among the four PAHs (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene), chrysene was the most abundant compound (Table 2). As a whole, chrysene and benzo (b)fluoranthene were detected in more than 60% of the samples, while benzo[a]pyrene, known to be the most carcinogenic, was found in only 4 samples. Chrysene was present in 67% of the wedge clam samples (from 0.41 to 1.10 ng/g ww) while benzo[a]pyrene was found in 20% of the analyzed samples with (mean 1.30 ng/g





**Figure 1.** Individual levels of PAHs (ME±SD) in wedge clams (*D. trunculus*) sampled from the northern and southern locations of the Bulgarian Black Sea coast.

ww). Benzo[k]fluoranthene was found (mean 0.56 ng/g ww) only in wedge clams from Shkorpilovtsi (S3), Sveti Vlas (S8), Sozopol (S14) and Cape Emine (S16) (Table 2).

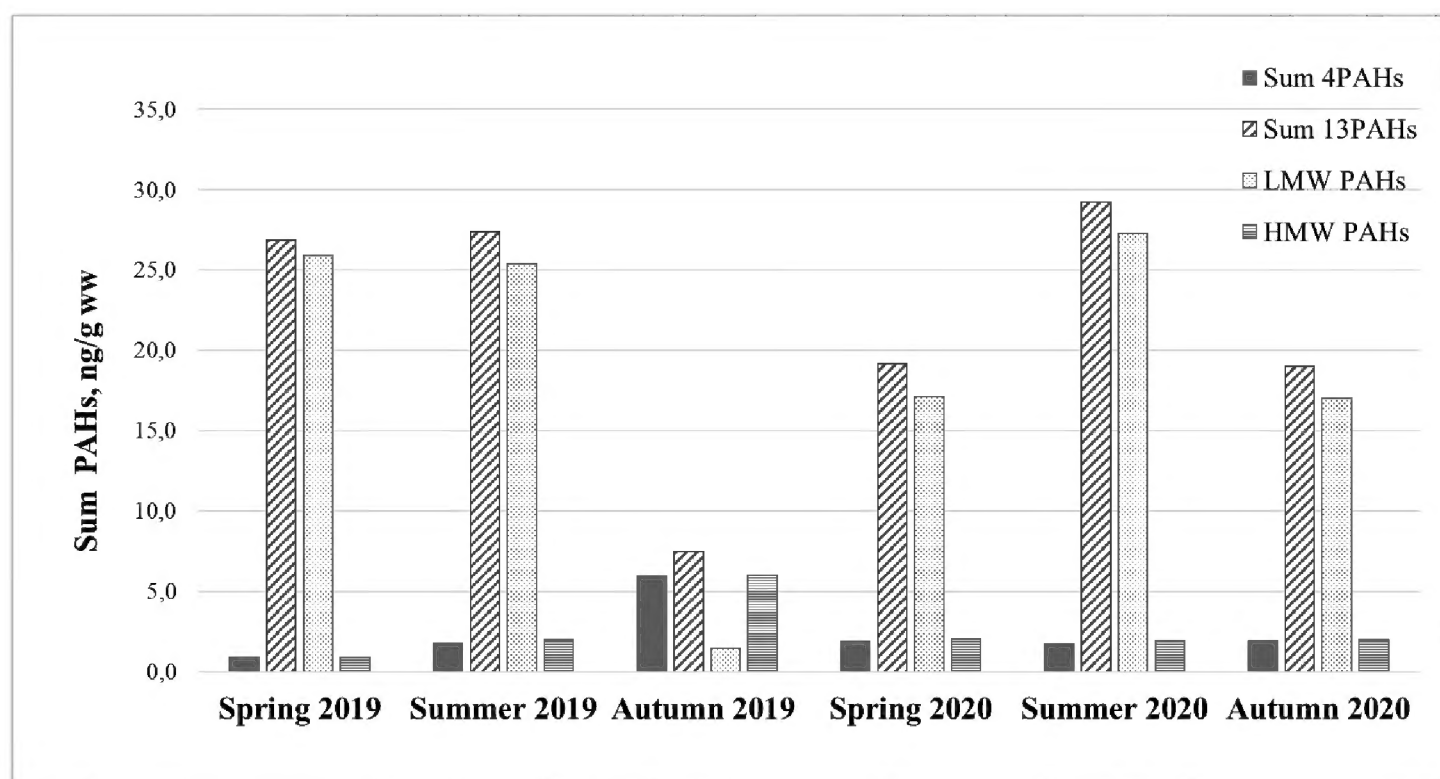
The mean individual levels of PAHs in *D. trunculus* sampled from the northern (north of Cape Emine) and southern (south of Cape Emine) locations of the Bulgarian Black Sea coast are presented in Fig. 1.

In general, the mean concentrations of CHR, BbFA, and BaP were higher in wedge clams from the northern coastal zone (Fig. 1). The concentration of PHE in wedge clam samples from locations in the northern part of the Black Sea coast was significantly higher (23.44 ng/g ww) than from the southern coast samples (17.00 ng/g ww). Only the mean concentration of FL was significantly higher in wedge clams from the southern coastal locations, compared to the northern ones.

The level of PAHs, accumulated in the studied wedge clams, displayed some seasonal variation (Fig. 2). The Sum PAH<sub>4</sub> was significantly higher in autumn (difference significant for autumn 2019) and Sum PAH<sub>13</sub> was significantly higher in summer compared to spring (2020) and autumn (2019 and 2020). The low molecular weight (LMW) PAHs accumulation was lower in autumn, whereas the accumulated high molecular weight (HMW) PAHs were higher in autumn (difference significant for autumn 2019).

## Discussion

The results obtained in this study showed that in all wedge clams different PAHs were accumulated. This is a strong indication that seawater and sediments along the Bulgarian Black Sea coast are contaminated with organic pollutants. Similar findings for the Black Sea were



**Figure 2.** Total PAHs levels in wedge clams (*D. trunculus*) sampled in different seasons from the studied locations off the Bulgarian Black Sea coast.

reported earlier for bivalves from the Crimean Peninsula (Olenycz et al. 2015), Sevastopol Bay (Shchekaturina et al. 1995) and the Turkish coast (Güven and Coban 2012).

PAHs can originate from a variety of sources: LMW PAHs are defined as petrogenic compounds (resulting from spillage of diesel and fuel oil), and HMW PAHs as having pyrolytic origin (products of the incomplete combustion of organic matter) (Srogi 2007; Mercogliano et al. 2016). The molecular ratio of LMW and HMW hydrocarbons indicates the sources of PAHs. In our study the ratio LMW/HMW PAHs was higher than one (mean 17.61), suggesting that PAHs pollution of the Bulgarian Black Sea coastal region was predominantly of petrogenic origin. The pattern of accumulated composition of PAHs established by us was as follows: 91% were LMW PAHs with 3-rings (PHE and FL); 8.7% were PAHs with 4- and 5-rings -; < 1% were the PAHs with 6-rings.

In this study, the highest levels of Sum PAH13 were found in wedge clams from Tsarevo (S17), followed by Arkutino (S12) and Nessebar (S11). Given that the PAHs have mainly pyrolytic and petrogenic origin (Mercogliano et al. 2016), it seemed most likely that the high content of PAHs, present in wedge clams from these locations, resulted from local spills or currents that carried these pollutants. Relatively high levels of Sum PAH13 were also found in the wedge clams from the southern locations Duni (S13) and Arkutino (S12), but they were most probably the result of the oil spill in 2018 from the sunken ship SS Mopang near the coast of Sozopol (Panteleeva et al. 2021). This was further confirmed by the high ratio LMW/HMW PAHs established in *D. trunculus* from these locations, which strongly suggested a petrogenic source of the pollution. Although all the mentioned localities are situated in the southern part of the Bulgarian Black Sea coast, there were no significant differences present in the

accumulated PAHs in wedge clams from the northern and southern coastal regions. As an exception, statistically higher levels of accumulated phenanthrene were measured in wedge clams from the northern coastal areas. This could be associated with discharges from the Danube River, containing degraded petroleum and fresh oil which are reported as the major contributor of total hydrocarbons (Readman et al. 2002).

The high content of LMW PAHs in the studied wedge clams, strongly indicated that oil pollution was the major source of contamination. Phenanthrene has high lipophilicity that makes it readily absorbed from the gastrointestinal tract and transferred to the tissues (Ifegwu and Anyakora 2016). The highest benzo[a]pyrene content was observed in wedge clams from Varna Bay (S5), indicating significant ecological pressure from the intensive maritime traffic and industrial activities in the area.

Most published data on PAHs accumulation concerned mussels (*M. galloprovincialis*) (Perugini et al. 2007; Mercogliano et al. 2016) and the only more comprehensive study of PAHs in wedge clams was from the Mediterranean Sea (Ferrante et al. 2018). The content of PAHs in *D. trunculus* found in our study (4.75 ng/g ww) was significantly lower than the PAHs' levels in the wedge clams reported from the Mediterranean Sea (247.4 ng/g ww) (Ferrante et al. 2018).

Admissible limit was set for Sum PAH4 in bivalve species by EC n.835 regulation (EC 2011). The established in our study values of Sum PAH4 in wedge clams were well below the regulation limit (35 ng/g ww). With regard to benzo[a]pyrene, which is considered to be highly carcinogenic according to the International Agency for Research on Cancer (IARC Group 1) (IARC 2010), we also did not establish values exceeding the admissible limits (6 ng/g ww) (EC 2011).

## Conclusion

Results from this study showed the presence of accumulated PAHs in wedge clams from all the 15 locations along the Bulgarian Black Sea coast, which strongly indicated the presence of contamination with organic pollutants. The level of PAHs content in the wedge clams showed variations depending on local conditions. The maximum PAHs content was found in wedge clams from locations near to urbanized and industrial areas, or areas with highly intensive tourism. In general, there were no significant seasonal variations in the level of total PAHs, although some exceptions were observed. Our data did not show the presence of PAHs values exceeding admissible limits set by national and EC regulations. The data from the present research can be useful in further studies for assessing PAHs pollution levels and risks for human exposure using *D. trunculus* as bioindicator species.

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## References

- Banni M, Negri A, Dagnino A, Jebali J, Ameer S, Boussetta H (2010) Acute effects of benzo[a]pyrene on digestive gland enzymatic biomarkers and DNA damage on mussel *Mytilus galloprovincialis*. *Ecotoxicology and Environmental Safety* 73(5): 842–848. <https://doi.org/10.1016/j.ecoenv.2009.12.032>
- Barhoumi B, El Megdiche Y, Clérandeau C, Ameer WB, Mekni S, Bouabdallah S, Derouiche A, Touil S, Cachot J, Driss MR (2016) Occurrence of polycyclic aromatic hydrocarbons (PAHs) in mussel (*Mytilus galloprovincialis*) and eel (*Anguilla anguilla*) from Bizerte lagoon Tunisia and associated human health risk assessment. *Continental Shelf Research* 124: 104–116. <https://doi.org/10.1016/j.csr.2016.05.012>
- EC (2011) Commission Regulation (EC) n.835/2011 of 19 August 2011 amending. 2011n. Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Official Journal of the European Union*, L 215: 4–8.
- Ferrante M, Zanghi G, Cristaldi A, Copat C, Grasso A, Fiore M, Signorelli SS, Zuccarello P, Oliveri Conti G (2018) PAHs in seafood from the Mediterranean Sea: An exposure risk assessment. *Food and Chemical Toxicology* 115: 385–390. <https://doi.org/10.1016/j.fct.2018.03.024>
- Georgieva S, Stancheva M, Makedonski L (2016) Investigation about the presence of organochlorine pollutants in mussels from the Black Sea Bulgaria. *Analele Universitatii Ovidius Constanta. Seria Chimie* 27(1): 8–12. <https://doi.org/10.1515/auoc-2016-0006>
- Gumus MR, Todorova VR, Panayotova MD (2020) Recent Observations on the Size Structure of *Donax trunculus* Linnaeus. 1758 and *Chamelea gallina* (Linnaeus. 1758) in the Bulgarian Black Sea as Status Indicators of Commercially Exploited Shellfish under the Marine Strategy Framework Directive (MSFD). *Ecologia Balkanica. Special Edition* 3: 63–71. [http://web.uni-plovdiv.bg/mollov/EB/2020\\_SE3/063-071\\_eb.20SE310.pdf](http://web.uni-plovdiv.bg/mollov/EB/2020_SE3/063-071_eb.20SE310.pdf)
- Güven K, Coban B (2012) Oil pollution in Turkish Black Sea coast and input of oil from Turkey to the Black Sea in 2004–2007. *Fresenius Environmental Bulletin* 21: 3711–3717.
- IARC (2010) Monographs on the evaluation of carcinogenic risks to humans. some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. Lyon. <http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf>
- Ifegwu OC, Anyakora C (2016) Chapter Six - Polycyclic Aromatic Hydrocarbons: Part II, Urine Markers In: Makowski GS (Ed.) *Advances in Clinical Chemistry* vol. 75. Elsevier, 159–183. <https://doi.org/10.1016/bs.acc.2016.03.001>
- Kucuksezgin F, Gonul LT, Pazi I, Ubay B, Guclusoy H (2020) Monitoring of polycyclic aromatic hydrocarbons in transplanted mussels (*Mytilus galloprovincialis*) and sediments in the coastal region of Nemrut Bay (Eastern Aegean Sea). *Marine Pollution Bulletin* 157: e111358. <https://doi.org/10.1016/j.marpolbul.2020.111358>
- Lehtonen K, d'Errico G, Korpinen S, Regoli F, Ahkola H, Kinnunen T, Lastumäki A (2019) Mussel Caging and the Weight of Evidence Approach in the Assessment of Chemical Contamination in Coastal Waters of Finland (Baltic Sea). *Frontiers in Marine Science* 6: e688. <https://doi.org/10.3389/fmars.2019.00688>
- Machado AA, Hoff ML, Klein RD, Cordeiro GJ, Lencina Avila JM, Costa PG, Bianchini A (2014) Oxidative stress and DNA damage responses to phenanthrene exposure in the

- estuarine guppy *Poecilia vivipara*. Marine Environmental Research 98: 96–105. <https://doi.org/10.1016/j.marenvres.2014.03.013>
- Mercogliano R, Santonicola S, De Felice A, Anastasio A, Murru N, Ferrante MC, Cortesi ML (2016) Occurrence and distribution of polycyclic aromatic hydrocarbons in mussels from the gulf of Naples. Tyrrhenian Sea. Italy. Marine Pollution Bulletin 104(1–2): 386–390. <https://doi.org/10.1016/j.marpolbul.2016.01.015>
- Okay O, Karacik B, Güngördü A, Yılmaz A, Koyunbaba NC, Yakan SD, Henkelmann B, Schramm K-W, Ozmen M (2017) Monitoring of organic pollutants in marine environment by semipermeable membrane devices and mussels: Accumulation and biochemical responses. Environmental Science and Pollution Research International 24(23): 19114–19125. <https://doi.org/10.1007/s11356-017-9594-0>
- Olenycz M, Sokołowski A, Niewińska A, Wołowicz M, Namieśnik J, Hummel H, Jansen J (2015) Comparison of PCBs and PAHs levels in European coastal waters using mussels from the *Mytilus edulis* complex as biomonitors. Oceanologia 57(2): 196–211. <https://doi.org/10.1016/j.oceano.2014.12.001>
- Panteleeva M, Chamova R, Radeva N, Romanova H (2021) Anthropogenic Disasters on Bulgarian Territory: Chemical Accidents on Land and at Sea. Journal of IMAB—Annual Proceeding Scientific Papers 27(2): 3718–3722. <https://doi.org/10.5272/jimab.2021272.3718>
- Perugini M, Visciano P, Giammarino A, Manera M, Di Nardo W, Amorena M (2007) Polycyclic aromatic hydrocarbons in marine organisms from the Adriatic Sea. Italy. Chemosphere 66(10): 1904–10. <https://doi.org/10.1016/j.chemosphere.2006.07.079>
- Readman JW, Fillmann G, Tolosa I, Bartocci J, Villeneuve JP, Catinni C, Mee LD (2002) Petroleum and PAH contamination of the Black Sea. Marine Pollution Bulletin 44(1): 48–62. [https://doi.org/10.1016/S0025-326X\(01\)00189-8](https://doi.org/10.1016/S0025-326X(01)00189-8)
- Shchekaturina TL, Khesina AL, Mironov OG, Krivosheeva LG (1995) Carcinogenic polycyclic aromatic hydrocarbons in mussels from the Black Sea. Marine Pollution Bulletin 30(1): 38–40. [https://doi.org/10.1016/0025-326X\(94\)00070-P](https://doi.org/10.1016/0025-326X(94)00070-P)
- Srogi K (2007) Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: A review. Environmental Chemistry Letters 5(4): 169–195. <https://doi.org/10.1007/s10311-007-0095-0>
- Suárez P, Ruiz Y, Alonso A, San Juan F (2013) Organochlorine compounds in mussels cultured in the Ria of Vigo: Accumulation and origin. Chemosphere 90(1): 7–19. <https://doi.org/10.1016/j.chemosphere.2012.02.030>
- Tanabe S, Subramanian A (2006) Bioindicators Suitable for Monitoring POPs in Developing Countries. Kyoto University Press, Kyoto, 190 pp.
- Tlili S, Mouneyrac C (2019) The wedge clam *Donax trunculus* as sentinel organism for Mediterranean coastal monitoring in a global change context. Regional Environmental Change 19(4): 995–1007. <https://doi.org/10.1007/s10113-018-1449-9>